Phosphorus Fractions in Arable and Mountain Soils and their Humic Acids

Gabriela BARANČÍKOVÁ[1], Tibor LIPTAJ[2] and Nadežda PRÓNAYOVÁ[2]

[1]*Soil Science and Conservation Research Institute in Bratislava, regional station Prešov, Slovak Republic;* [2]*Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Bratislava, Slovak Republic*

Abstract: Soil phosphorus (P) can exist in various inorganic (Pi) and organic forms (Po). Specific determination of Pi can be obtained by fractionate methods. However, the discrimination between the chemically different forms of Po in soil is not simple. Nowadays, the method of choice for the determination of various soil inorganic and organic P compounds is ^{31}P NMR. In this paper, the determination of various phosphorus forms (available P, total P, Pi and Po fractions) in arable and mountain soils is presented. Besides, the detailed characterisation of P compounds in humic acids (HA) is also shown. The results obtained show that the highest content of the available P can be found in arable soils with a high input of fertilisers, and that the predominant part of Pi is included in hardly soluble fractions, mainly in the soil types with neutral soil reaction. Our data also show the correlation between total P and Po, the dominant form of P in the topsoil of mountain soils. Phosphomonoesters represent the major types of P in HA structure. The correlations between phosphomonoesters of type I and some humification parameters of HA and qualitative parameters of soil organic matter suggest that higher amounts of more recalcitrant monoesters can be found in more mature soil organic matter with a higher humification degree.

Keywords: inorganic P; organic P; soil types; humic acids; ^{31}P NMR

Microbial activity plays a major role in the nutrient turnover in general and in P transformation and redistribution into different inorganic and organic forms in particular (Stewart & Tiessen 1987). Native phosphorus in soils is derived mainly from apatite. During its weathering and soil development, phosphorus is liberated and:
– adsorbed by plants and recycled,
– incorporated into the organic mater of soils and sediments,
– redeposited as either insoluble or slowly soluble mineral forms.

Only a small fraction of phosphorus occurs in water soluble forms. Approximately 90 percent of the soil phosphorus occurs in insoluble or soluble but fixed forms (primary phosphate minerals, humus P, Ca, Fe, and Al phosphates, phosphates fixed by colloidal oxides and silicate minerals). Various soil phosphorus forms can be differentiated also on the basis of their availability for the plants as available or non-available P fractions: (a) P in soil solution, (b) adsorbed P (labile) localised on the cover of soil colloids, (c) precipitable P (occluded P) from soil solution (part of this P form is also labile), (d) fixed P, E – P immobilised in organic soil phosphates (Haman 1980).

The most common forms of P found in soils are the inorganic pools (Pi) orthophosphate, inorganic

polyphosphate and pyrophosphate, and the organic compounds (Po) polyphosphate, phosphonate, orthophosphate monoester and orthophosphate diester. Among the organic compounds, monoesters are certainly predominant (Cardoso *et al.* 2003).

The most important for farmers is the P, which is available for plants. The available P is the basic parameter which determines the nutrients supply in the Soil Monitoring System. Some information about the bound forms of inorganic P can be obtained by the fractionate methods. One of these methods – Scharafat method (Scharafat 1970) is used also in Slovakia Soil Monitoring (Kobza *et al.* 2002). However, chemical composition of organic P in soil is not easy to determine (Preston 1996; Cardoso *et al.* 2003). For this reason, only the total organic P is determined according to Dean Method in the Soil Monitoring System.

In Slovakia soil types, the proportion of organic phosphorus in the total phosphorus is 23–45% (Hrtánek 1994). For many soils, a high proportion of phosphorus exists in organic forms that are difficult to analyse by chemical techniques. Phosphorus-31 NMR spectroscopy has been successfully employed for the identification of a wide range of organic and inorganic P compounds in solution (Newman & Tate 1980; Dougherty *et al.* 2005). This technique offers an alternative approach and is able to quantify relative amounts of P in different chemical fractions (orthophosphate, phosphate mono and di-esters, phosphonate, and polyphosphate) (Preston 1996).

Although present in low concentrations in humic substances, ^{31}P is highly sensitive nucleus and a great scope exists for NMR studies with this nucleus. While P content in soil humic acids is relatively low (800–1500 mg/kg) its considerable part belongs to the organic P. ^{31}P NMR spectra of HA can provide valuable information about P in soil (Novák *et al.* 2005).

In this paper, the differences between the total phosphorus content and the contents of inorganic and organic P forms in mountain and arable soils have been evaluated. Particularly, different structural types of P in HA isolated from mountain and arable soil types determined by ^{31}P NMR technique are demonstrated.

MATERIAL AND METHODS

Phosphorus fractions were isolated from the soil samples coming from 3 key monitoring localities of mountain and 4 localities of arable soils. From these soil samples humic acids were also isolated. The soil types were classified according to the WRB 2006 system (WRB 2006). Basic characteristics of the soils are presented in Table 1.

The available forms of P (Pavail) were determined according to Egner. Easily and hardly soluble inorganic phosphorus (fractions Pi-E, Pi-H) were determined after Scharafat and total organic phosphorus with the method by Dean (Kobza *et al.* 1999).

Fractionation of humus and the calculation of the carbon ratio of humic and fulvic acids (Cha/Cfa) and optical parameter Q^4_6 determination according to Kononovova and Belčikova method were done (the results are shown in Kobza *et al.* 1999).

Humic acids were isolated using the IHSS method (Kobza *et al.* 1999). Particular characterisation of the chemical structure of these HA (elemental analysis (C H N), optical parameter ($E^{1\%}_6$), aromatic carbon (Car), and the degree of humification (α)

Table 1. Basic characteristics of soil localities

Locality	Soil types	pH/HCl	CaCO$_3$ (%)	Corg (%)	Nt (mg/kg)	< 0.01mm (%)
Donovaly M	Hyperskeletic Leptosol	6.9	18	13	11 900	35
Voderady A	Calcaric Chernozem	7.2	1.25	1.5	1 640	40
Spišská Belá A	Endogleyic Chernozem	6.3	0	2.2	2 000	39.2
Stakčín A	Luvic Stagnosol	6.5	0	1.4	1 695	42.1
Macov A	Mollic endogleyic Fluvisol	7.4	11	1.9	1 945	43.9
Chopok M	Umbric Hyperskeletic Leptosol	3.3	0	13	7 800	15
Sitno M	Umbric Andosol	4.2	0	10.5	8 660	27.8

M – mountain soil; A – arable soil

can be found in our previous papers (Barančíková 1999, 2002).

For NMR experiments, 100 mg of isolated HA samples were dissolved in 2 ml of deuterated water (Aldrich) and the pH of the solutions was adjusted to 9.5. All ^{31}P NMR experiments were run at 23°C on a Varian Unity-Inova 600 MHz spectrometer using basic one-pulse experiment with the following set of the acquisition parameters: spectrometer frequency 242.803 MHz; relaxation delay 1 s, acquisition time 1.6 s; excitation pulse flip angle 45°, spectral width 50 000 Hz and a continuous broadband decoupling of the protons. ^{31}P NMR chemical shifts were referenced to the signal of external 85% H_3PO_4 sealed in a capillary. Prior to Fourier transformation the accumulated data were apodized with exponential function (line broadening 10 Hz).

RESULTS AND DISCUSSIONS

Soil phosphorus fractions

Some soil characteristics like land use, soil type, parent material, climatic zone, etc. have been related to the content of soil phosphorus. For most soils, the availability of P is maximal in the slightly acid to neutral pH range (Marschner 1995). A considerably higher content of the available P was found in arable soils in comparison with mountain soils (Table 2). According to Linkeš *et al.* (1997), the high amounts of the available P (Pavail) in the main arable soil types in Slovakia indicates intensive fertilisation. High contents of Pavail

were found in our samples, but different in the individual soil types. Because the soil pH strongly affects the P availability, the lowest content of Pavail was detected in strongly acid mountain soils (Sitno, Chopok) (Table 2). A significant linear correlation between the amount of the available P and the soil pH was found (Table 4). The highest content of Pavail in arable soils was found in the Calcaric Chernozem and Mollic Fluvisol, while its contents in Stagnosol and Endogleyic Chernozem were substantially lower (Table 2). The level of the available P in mountain soils is considerable lower than in arable soils because of no input of P via fertilisation taking place there. The lowest content of the available P among all soil types examined was found in the Umbric Leptosol soil (Table 2).

The predominant part of inorganic phosphorus occurs in hardly soluble fractions, mainly in the soil types with neutral pH values. Considerably lower contents of easily soluble Pi were observed in the soil samples studied (Table 2). These findings are in agreement with the literature findings (Hrtánek 1994; Kobza *et al.* 2002). Significant linear correlations also exist between the contents of inorganic P fractions (Pi-H, Pi) and pH values (Table 4). Based on this evidence, it can be concluded that the content of inorganic P is significantly influenced by the soil pH value.

Organic P is often the dominant form in the topsoil of pasture and mountain soils. Its amount depends to a large extent on the organic matter content in the soil (Barber 1995). The average content of organic phosphorus in cultivated soils ranges from 5–50% of total P (Harison 1987). In

Table 2. Content of phosphorus fractions (mg/kg)

Locality	(Egner) Pavail	(Scharafat)			(Dean)		
		Pi-E	Pi-H	Pi	Po	Ptot	Po (% of Ptot)
Donovaly	46.3	17.6	457	474.6	1575	2049.6	76.8
Voderady	189.5	24	360	384	265	649	40.8
Spišská Belá	82	66.5	302.5	369	395	764	51.7
Stakčín	98.5	65.8	342	407.8	195	602.8	32.3
Macov	207.1	37.5	638	675.5	377.5	1053	35.8
Chopok	2.3	27.8	62	89.8	835	924.8	90.3
Sitno	8.5	25.5	283	308.5	1950	2258.5	86.3

Pavail – available phosporus; Pi-E – easily soluble fraction of inorganic phosphorus; Pi-H – hardly soluble fraction of inorganic phosphorus; Pi – inorganic phosphorus; Ptot – total phosphorus; Po – organic phosphorus

the arable soils investigated, the proponion of Po in Ptot ranges between 32–50% and is typical for the agricultural soils in Slovakia (Hrtánek 1994). Considerably higher proportions of Po were found in mountain soils (77–90%) with high contents of soil organic matter (Table 1, 2). A significant correlation between the organic phosphorus content and the total P content in soils was found by Harrison (1987). High contents of Po with high contents of Corg in the mountain soils types such as Hyperskeletic Leptosol and Umbric Hyperskeletic Leptosol were reported also by Kobza *et al.* (2002). Hrtánek (1994) found that a large fraction of the total soil phosphorus is organically fixed phosphorus whole high content was determined in topsoil with a high humus content. A correlation between Po and the content of soil humus was reported also by Kobza *et al.* (2002). Harrison (1987) found a relationship between the total organic P content of soils and their organic matter and nitrogen contents. Similarly, we also found a significant linear correlation between Po and Ptot, and Po, organic carbon, and total nitrogen contents in our samples (Table 4).

Determination of phosphorus by ^{31}P NMR in humic acids

The identification of the signals in ^{31}P NMR spectra in our work was done in accordance with the literature data (Ogner 1983; Adams & Byrne 1989; Zangh *et al.* 1999). It is well known that ^{31}P NMR chemical shifts of the phosphate group are sensitive to the variation in the pH value and ionic strength of the solution (Novák *et al.* 2005). Unfortunately, with the most literature data the conditions under which the spectra were collected are described very loosely and therefore the detailed specification of each signal resolved was not possible.

The typical ^{31}P NMR spectrum observed under the conditions described above with the assignment of the individual signals is shown in Figure 1. Well separated are the signals belonging to the phosphonate groups (16–20 ppm). The strongest signal belongs to phosphomonoesters (7–3 ppm), which is in agreement with the literature data (Cardoso *et al.* 2003). Their signals partially overlap the signal of inorganic orthophosphate. The degree of

Table 3. Contents of different structural types (in molar %) determined as the relative integral intensities in ^{31}P NMR spectra

Spectral region			Soil type					Endogleyic Chernozem		Mollic Fluvisol	
Compound type		ppm	Luvic Stagnosol	Calcaric Chernozem	Hyperskeletic Leptosol	Umbric Leptosol	Andosol	topsoil	subsoil	topsoil	subsoil
Phosphonates	I	24–22									
	II	21–18	0.47	1.03	4.7	8.47	1.38	3.25	3.26	1.73	1.64
	III	18–17	0.8	0.38	1.65	6.81	0.91	1.53	2.08	1.24	0.7
P_{ino}		7–5	21.88	28.4	21.41	18.86	31.95	19.68	18.66	21.68	25.61
Phosphate monoesters	I	5–4.2	23.12	29.16	24.26	23.6	29.1	24.78	24.34	32.43	33.32
	II	4.2–3.9	12.12	9.61	12.31	14.31	11.51	11.93	13.02	12.58	11.33
	III	3.9–3.2	18.37	14.28	15.87	19.73	16.57	17.03	16.37	15.62	13.38
Phosphate diesters	I	2.4–0.5	6.28	5.85	4.6	1.93	2.85	7.32	5.94	3.94	3.57
	II	0.5–(−0.5)	11.61	8.74	12.41	5.8	2.86	11.41	12.7	10.28	9.32
	III	(−0.5)–(−2)	5.35	2.55	2.79	0.49	2.87	3.07	3.63	0.5	1.13

Table 4. Correlation coefficient *R* for significant correlations between soil properties, properties of soil organic matter and P forms

	Pavail	Pinorg-H	Pinorg	Porg	Pm I	Pd II
pH/KCl	0.81*	0.84**	0.85**			0.83**
Corg				0.82*		
Nt				0.89**		
Ptot				0.97**		
Chk/Cfk					0.8*	
Q_6^4					0.8*	
α					0.8*	
Car					0.74*	
$E_6^{1\%}$					0.87**	
C					0.87*	

*95% level of significance; **99% level of significance; Pm I – monoester in spectral region 5–4.2 ppm; Pd II – diester in spectral region 0.5–(−0.5) ppm; Cha/Cfa – carbon ratio of humic and fulvic acids; Q_6^4 – optical parameter of HA (ratio of optical densities measured at 460 and 650 nm); α – degree of humification (%) (Car + Caliph)/Car; Car – aromatic carbon of HA determined by ^{13}C NMR; $E_6^{1\%}$ – the absorbance coefficient E for 1% HA solution at 600 nm; C – carbon of HA (wt%)

spectral overlap and spectral resolution changes with the soil type from which HA is isolated and depends on its composition. According TURNER *et al.* (2003) and CONDRON *et al.* (1985), the dominant amounts of the signals in this region come from inositol (myo-inositol hexakisphosphate) and cholin phosphates. The shape of the spectral signal (dominant narrow singlets characteristic for the subspectra of these compounds) strongly supports this proposal. Orthophosphate diesters resonate at higher field (~ 0 ppm). They provide several resolved signals, however, no dominant individual component can be identified with some specific compound. This spectral region can be divided into several parts belonging to different structural types: sugar diesters that resonate at higher frequency (3.5–0.6 ppm), and orthophosphate diesters (0.6–(−0.5) ppm) (MAHIEU *et al.* 2000). Diesters are considered to be chemically more labile than monoesters and are more acces-
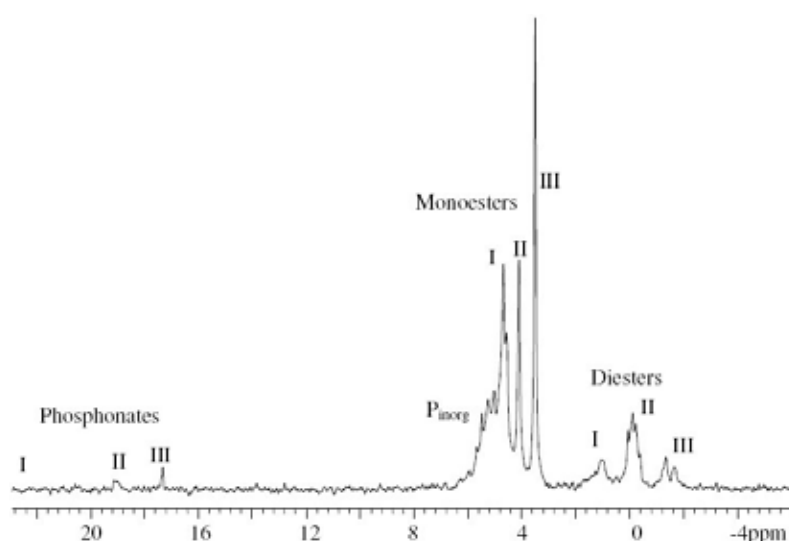

Figure 1. Typical ^{31}P NMR spectrum of HA sample (Luvic Stagnosol) with the assignment of the individual resonances in agreement with the assignment in the Table 3

sible to microbial and enzyme attacks (Cardoso *et al.* 2003). Pyrophosphates and polyphosphates can be easily distinguished in ^{31}P NMR spectra as they have well separated resonances at ~ –5 ppm and –20 ppm, respectively. However, no such signal was observed in the samples studied, which means that their contents were below the sensitivity limit of NMR method.

In all analysed humic acids isolated from arable and mountain soils, the contents of phosphate monoesters varies only slightly (53–60%) and are several times higher in comparison to the contents of phosphate diesters (Table 3). Our findings are consistent with the findings of other authors (Mahieu *et al.* 2000; Cardoso *et al.* 2003; McDowell & Stewart 2006; He *at al.* 2006). Condror *et al.* (1985) reported that orthophosphate monoesters constitute the largest proportion of the P detected by NMR. Alkali extractable monoesters include inositol phosphates which are strongly connected to soil particles. Hence, monoesters are protected by sorption to soil organic matter and represent more recalcitrant forms then diesters. Phosphate monoesters of type I (5–4.2 ppm), which represent the highest content of monoesters (Table 3), are related to the degree of humification. We found significant linear correlations between the content of carbon in HA, optical properties of HA ($E^{1\%}_6$), the degree of humification represented by α parameter (ratio of aromatic and aliphatic carbon/ aromatic carbon), and the content of monoester I (Pm I) (Table 4).

Also Mahieu *et al.* (2000) found linear correlations between the monoester P content and the humification properties of HA. Relations between the organic forms of C and P were reported also by Preston (1996). Phosphate monoester I of HA was also correlated with other qualitative parameters which are used to characterise the soil organic matter (Cha/Cfa), optical parameter (Q^4_6), however, no correlations was found between P fractions in HA and the soil organic carbon content. It seems that the most common P fractions in HA depend mainly on the quality of the soil organic matter and not on the total Corg in the soil. It can be concluded that higher amounts of the more recalcitrant monoesters can be found in more mature soil organic matter with a higher humification degree.

We found relatively great differences in the relative content of diesters (8–23%) in the HA samples studied. In HA isolated from the soil types with

neutral pH, two times higher content of diesters was observed then in HA isolated from acid mountain soils (Umbric Leptosol, Umbric Andosol) (Table 3). McDowell and Stewart (2006) showed that the labile organic P forms (diesters) were depleted in forest soils. We found a significant correlation between diesters contents (mainly diester II (spectral region 0.5–(–0.5)) and the soil pH value (Table 4). McDowell and Stewart (2006) reported that diesters are mostly made up of compounds such as DNA and have a lower charge density since the phosphate group is bound to C by two ester linkages, which means that they are less able to interact ionically as compared to monoesters with only one covalent bonding to the C-moiety. Condron *et al.* (1985) reported that diester P compounds are readily mineralised in soil, which could account for their small quantities found in HA. Also Briceno *et al.* (2004) confirmed that the diester P is a more easily mineralised form of organic P in soils than the monoester P.

The soil depth (topsoil, subsoil) has no important influence on the distribution of different P fractions in Endogleyic Chernozem and Mollic Endogleyic Fluvisol. The compositions of P compounds in HA isolated from topsoil and subsoil of Endogleyic Chernozem and Mollic Endogleyic Fluvisol are approximately identical (Table 3). A similar pattern was observed in the ^{31}P NMR spectra from the samples prepared from the soil taken at the depths between 0–15 cm and 15–30 cm (Briceno *et al.* 2004).

The contents and compositions of various phosphorus compounds in different soil types are very variable and depend mainly on the soil properties and soil use. The highest content of the available P was found in arable soils. Concerning inorganic phosphorus, this form of soil P is included in hardly soluble fractions, mainly in the soil types with neutral soil reaction. Our findings confirmed that organic P represents a substantial part of the total P in mountain soils with a high content of soil organic matter, where Po content ranges between 76–90% of the total P content.

^{31}P NMR spectra of humic acids isolated from the investigated soils showed that phosphorus-31 nuclear magnetic resonance spectroscopy, which has been used to study the distribution of different P fractions in humic acids is a simple and direct method. Our data confirmed the previous finding that most of the organic P detected by NMR was in monoester fractions. We found a

significant correlation between the most abundant monoester fraction (MP I) and the humification degree of HA. Based on the correlations between MP I and the qualitative parameters of soil organic matter, it can be concluded that the more recalcitrant monoester P fractions occur in more mature soil organic matter with a higher degree of humification.

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Corresponding author:

RNDr. Gabriela Barančíková, CSc., Výskumný ústav pôdoznalectva a ochrany pôdy Bratislava, regionálne pracovisko Prešov, Raymannova 1, 08001 Prešov, Slovenská Republika
tel.: + 421 517 731 054, fax: + 421 517 723 184, e-mail: bar@vupop.sk